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Aspergillus fumigatus Keratitis with Intraocular Invasion in 15-Day-Old Chicks

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SUMMARY. Eye infections were initially observed in single-comb white leghorn breeder chicks at 5 days of age, and morbidity increased from 0.05% to 1.5% after debeaking at 7 days of age. All chicks necropsied at 15 days of age had cheesy yellow exudate within the conjunctival sac of one eye and small (1 mm diameter) white nodular lesions in lungs and on thoracic air-sac membranes. Histopathologic examination of the eyes revealed septate fungal hyphae and inflammatory cells in the anterior chamber, cornea, and conjunctival sac. Similar fungal hyphae were present within lung granulomas. *Aspergillus fumigatus* was isolated from the eyes. Eye infections were the only health problem reported for several consecutive flocks on this farm. Elimination of moldy feed from the diet and environment and proper management of sawdust litter have prevented fungal ophthalmitis in subsequent flocks.

RESUMEN. *Reporte de Caso*—Queratitis con invasión intraocular por Aspergillus fumigatus en pollos de 15 días de edad.

Inicialmente se observaron infecciones oculares en reproductoras leghorn blancas de cresta sencilla a los 5 días de edad. La morbilidad aumentó de 0.05% a 1.5% luego del despique a los 7 días de edad. Todas las aves necropsiadas a los 15 días de edad contenían un exudado caseoso amarillo dentro del saco conjuntival de un ojo y lesiones nodulares blancas y pequeñas (1 mm de diámetro) en los pulmones y en las membranas de las sacos aéreos torácicos. El examen histológico de los ojos reveló la presencia de hifas micóticas septadas y de células inflamatorias en la cámara anterior, en la córnea y en el saco conjuntival. Se encontraron hifas similares en los granulomas del pulmón y se aisló *Aspergillus fumigatus* de los ojos. El único problema reportado consecutivamente en varios lotes en esta granja fué la presencia de infecciones oculares. La eliminación del alimento contaminado con hongos y el adecuado manejo de la cama de viruta de madera previno la oftalmitis micótica en los lotes siguientes.

Mycotic disease, such as aspergillosis, is still a threat to poultry health because of the warm, humid environment present in a floor brooder house. Outbreaks of disease due to various species of *Aspergillus* are common in chicks and poults (15). *Aspergillus fumigatus* is the species most commonly isolated from poultry. However, *A. flavus* and other species have also been reported from both chickens and turkeys (1,12,15). Aspergillosis in very young chicks and poults is usually associated with overwhelming exposure to large numbers of conidia from heavily contaminated feed, litter, or the hatchery environment (4,8,15,23,24). Infection of a variety of tissues by *Aspergillus* sp. is well documented (15).

Early clinical signs of conjunctival infections include turbid ocular discharge with periorbital swelling and edema. As the disease progresses, eyelids become swollen and may be adhered together, a keratitis develops, and a cheesy pellet of fibrinopurulent exudate develops beneath the nictitating membrane (20).

Invasion of the anterior chamber of the eye via the cornea by *A. fumigatus* is an unusual manifestation of mycotic ophthalmic disease

(13,19,20). The present report documents mycotic keratitis with intraocular invasion caused by *A. fumigatus* in 15-day-old breeder chicks.

CASE REPORT

Case history. Eight live 15-day-old chicks from a breeder farm were presented for necropsy on September 15, 1992. Eye infections, similar to those present in previous flocks on this farm, were initially observed in about 0.05% of the flock at 5 days of age. Morbidity increased to approximately 1.5% after chicks were debeaked and brooder guards were taken down to release the birds onto sawdust litter at 7 days of age. This flock contained 13,000 chicks—11,500 females and 1,500 males. No aspergillosis occurred in chicks on other farms hatched from the same facility before, after, or on the same day as the chicks in this report.

Necropsy. Gross lesions were observed in the eyes (Fig. 1), lungs, and thoracic air sacs. Each of the eight chicks submitted had unilateral periorbital swelling, turbid discharge from the eye, eyelids that were swollen and adhered together, cloudy cornea, and cheesy yellow exudate within the conjunctival sac. None had bilateral eye lesions. Small, white nodular lesions (1 mm diameter) were present also in the lungs and thoracic air sacs.

Histopathologic examination. Portions of lung, brain, and bursa of Fabricius from all chickens were fixed in 10% neutral buffered formalin and processed routinely for light microscopy. Four-micron-thick paraffin-embedded sections from each tissue were cut and stained with hematoxylin and eosin (H & E). Additional sections of the lungs were stained by Grocott's modification of Gomori's methenamine silver method. Eyes from selected chickens were collected at necropsy and fixed in Bouin's solution. Following fixation, globes were thoroughly washed in running tap water and 70% ethanol and trimmed. A mid-sagittal slab from each eye was embedded in paraffin, sectioned at 4 μm, and stained with H&E. Duplicate slides were stained with periodic acid–Schiff (PAS).

Microscopic examination revealed abnormalities in eyes and lungs. Corneas showed total loss of surface epithelium, and the stroma was necrotic and had focal, often confluent granulomas (Fig. 2). Numerous branching, septate fungal hyphae were present in most corneal granulomas and within conjunctival sac and anterior chamber exudate (Fig. 3). Granulomas consisted of an eosinophilic acellular mass surrounded by a rim of epithelioid macrophages, multinucleated giant cells, and heterophils (Fig. 4). Inflammatory cells in the conjunctival and anterior chambers consisted of numerous heterophils embedded in fibrin. The lens, retina, uvea, vitreum, optic nerve, and sclera did not contain significant lesions.

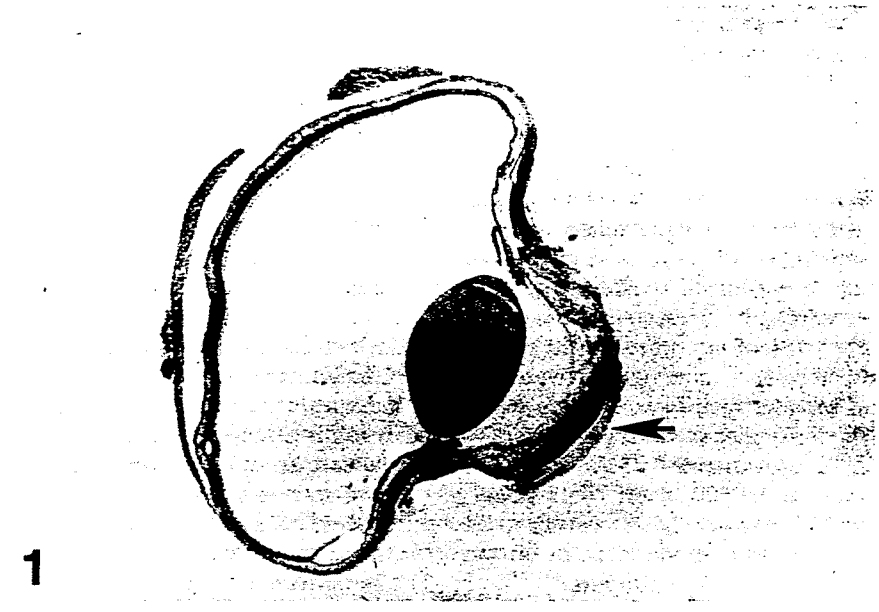
Both lungs in each of the eight chicks examined contained multiple granulomas within the lumina of tertiary bronchioles. The center of these lesions consisted of a small number of heterophils associated with fungal hyphae. Silver-stained sections showed that the hyphae were septate and branching. Densely packed epithelioid cells and multinucleated giant cells encircled the fungal hyphae and heterophils. The periphery of the lung lesions consisted of loosely arranged macrophages separated by edema fluid.

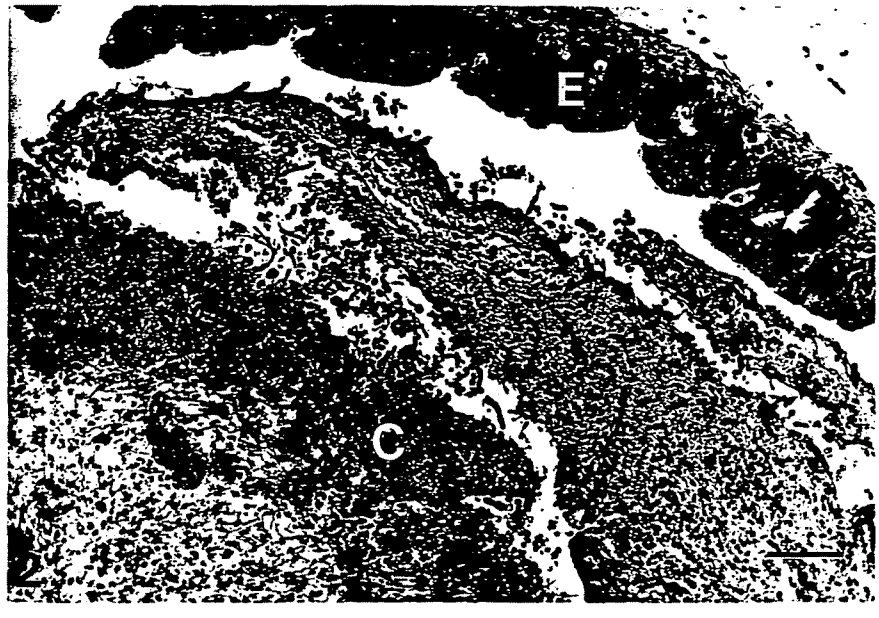
No brain lesions were detected, and the bursa of Fabricius was normal in all birds.

Microbiologic examination. The entire eye globe from selected birds was homogenized using a TenBroeck tissue grinder. Homogenized material was inoculated onto Czapeks (Difco, Detroit, Mich.), M40Y (14), Christensen malt salt (3), and Sabouraud dextrose agar. *A. fumigatus* was isolated in high numbers from all media and was the predominant fungal isolate. Slide cultures of the isolate on Czapek agar confirmed the identification of *A. fumigatus*.

DISCUSSION

Fungal ophthalmitis in chicks resulting from superficial invasion of the cornea is noteworthy. Fungal ophthalmitis commonly occurs after hematogenous or lymphatic dissemination of the organism from the respiratory epithelium to the posterior eye (15). If there is fungal invasion of the vitreous body, retina, or pecten, it is usually associated with respiratory aspergillosis in birds (13,16,17). Richard *et al.* (16,17,18) reported induction of lesions in the retina, pecten, and chambers of the eye 2 days or more after experimental aerosol exposure of turkey poults to viable *A. fumigatus* conidia. Because lesions were prominent in the pecten—a highly vascular organ of the avian eye—hematogenous dissemination of conidia from the respiratory

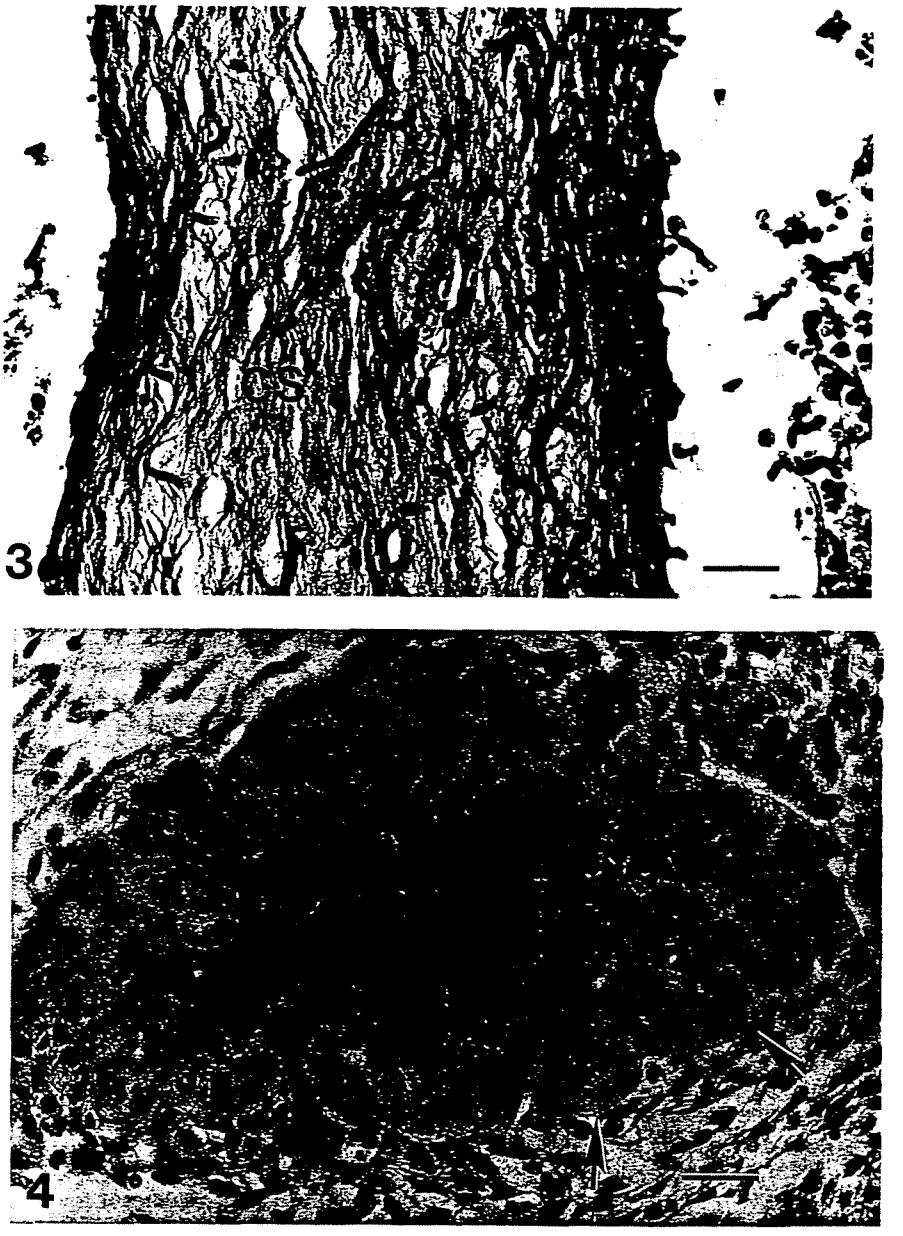




Figs. 1, 2. 1) Mycotic keratitis. Entire cornea is necrotic (arrow), but the posterior segment is devoid of any inflammatory exudate. H & E. 2) Necro-granulomatous exudate containing fungal hyphae between conjunctival epithelium (E) and the necrotic cornea (C). H & E. Bar = 15 μm.

tract to the eye was proposed (18). Lack of involvement of any intraocular structures in these chicks rules out hematogenous spread from respiratory lesions. In general, hematogenous spread of infection from the lung leads to mycotic endophthalmitis rather than keratitis.

The most likely pathogenesis for mycotic keratitis is that the cornea was initially damaged by fumes or ammonia in the bird's environment, leading to corneal epithelial erosions or superficial keratitis (25). Colonization of conjunctival surfaces with viable fungal elements from en-



Figs. 3, 4. 3) Fungal hyphae are widely dispersed throughout the corneal stroma (CS) but are most numerous in superficial corneal stroma. PAS. Bar = 5 μm. 4) Granuloma within corneal stroma. Epithelioid macrophages and giant cells (arrows) surround a necrotic center containing fungal hyphae. Bar = 5 μm.

vironmental sources may result in keratitis and conjunctivitis (9,15,19). Superficial infections may be limited to the cornea, conjunctiva, and nictitating membrane (9) or may invade to penetrate the anterior chamber and other internal structures of the eye (10,19). The presence of fungal hyphae and inflammatory cells in the anterior chamber of the eyes of these chicks is similar to lesions previously reported in turkeys and humans (13,19). The course of ocular in-

fection with *A. fumigatus* may be influenced by host and environmental factors (19,20) or by pathogenicity of the strain involved.

Increased susceptibility of young chicks to aspergillosis may be due to immaturity of phagocytes or to environmental factors. The host's defense against aspergillosis is primarily dependent upon phagocytes (2), and the number of phagocytes present in the respiratory tract of poultry is much lower than the number in the respiratory tract of mammals (5,21). Increased resistance of chicks to *Salmonella gallinarum* between 3 and 5 days posthatch is believed to be due to maturation of phagocyte bactericidal capability (6). Macrophage function could be further impaired if chicks are exposed to atmospheric ammonia, which inhibits phagosome–lysosome fusion in macrophages (7) and impairs mitogenic responsiveness of lymphocytes (11). Normally, inhaled conidia are encircled by respiratory macrophages within 15 minutes and lysosomes degranulate within 15 minutes (22).

The source of *A. fumigatus* conidia in the present case could not be determined with certainty. Even though infection occurred early in life, lack of evidence of aspergillosis in other flocks placed from the same hatchery tends to rule out the hatchery as the source of infection. Proper handling and storage of feed and new litter were suggested to the owners. A program of complete cleaning and disinfection of the brooder house between flocks was initiated. After placement of new hardwood litter and return of all equipment to the building, the brooder house was sprayed with imazalil (Clinafarm® EC; Pitman-Moore, Inc., Washington Crossing, N.J.). Since implementation of this program, no aspergillosis has occurred in successive flocks.

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